Post-CO_2 Enrichment Effects on Canopy Leaf Area Index

Edward Jordan

20150424

Masters of Forestry and Masters of Environmental Management

Executive Summary

This work is motivated by the IPCC climate forecast which states that carbon dioxide levels will increase to 550 ppmv by 2050. It is uncertain how forest ecosystems will respond to increases in carbon dioxide, what the potential for further carbon storage in forest ecosystems is, and how much these ecosystems can ameliorate anthropogenic carbon dioxide emissions. Previous research has yielded inconclusive answers to these questions, although certain trends began to emerge. For example, increases in atmospheric carbon dioxide allowed stands of several species to support higher leaf area index (e.g., aspen, birch and pine), while others (e.g., sweetgum) have shown a decline.

This work covers the last four years of the Free-Air Carbon Dioxide Enrichment (FACE) experiment conducted in the Blackwood Division of the Duke Forest. The experiment involved elevating carbon dioxide levels in four of the eight plots to +200 ppmv over ambient conditions during the first two of the four years (the last years of enrichment at the site), followed by two years under ambient CO_2. During the experiment, 11.2 g/m^2 nitrogen fertilization was added annually to half of each plot to determine effects of carbon dioxide enrichment based on site quality, which was moderately poor under native soil condition, and fertile with nitrogen addition. Species examined within the FACE site include loblolly pine, sweetgum and other broadleaved deciduous species. The working hypothesis was that leaf area index was enhanced by current year photosynthesis and is, therefore, dependent directly on carbon dioxide levels.

The methods employed to determine the effects of carbon dioxide on these species involved the collection of litter-fall monthly from January to September, and biweekly from October through December. The litter-fall thus collected was dried, sorted to species and weighed to obtain dry leaf mass. Further processing allowed estimation of fascicle sheath mass to total fascicle mass ratio, to facilitate removal from the former to estimate leaf area index, and a ratio of leaf area per mass to facilitate conversion of mass to area. Following, the seasonal dynamics of leaf area index were reconstructed using equations generated from Kinerson, et al. (1974) for loblolly pine, and Oren and Pataki (2001) for broadleaved deciduous species. Analysis of the data was performed on leaf area index enhancement ratios, to determine the effect of carbon dioxide on leaf area index on both moderate and high fertility sites.

Results showed that specific leaf area in pine does not respond to CO_2 concentration, while that of broadleaved deciduous species is likely to be higher in the future. In contrast, loblolly pine litter-fall mass trended to be higher under elevated CO_2, but that of broadleaved deciduous species was unaffected. The combined effect of these responses was to pine leaf area index under elevated atmospheric CO_2, as well as the index of the entire canopy, were influenced directly by current year's carbon dioxide levels. However, owing to greater variability relative to the mean, the enhancement of sweetgum leaf area index and that of other broadleaved deciduous species were not so clearly related to CO_2 enrichment. Loblolly pine leaf area index was increased 15% on moderate fertility sites and 7% on high fertility sites, while total canopy increased 13% on both moderate and high fertility sites. Interpretation of the results was further complicated by drought conditions in 2009, which affected the estimates of broadleaved deciduous species, creating an underestimation in the same year. Due to needle longevity, the drought-induced overestimates in 2009 created a corresponding underestimation of pine leaf area index in 2010.

In conclusions, leaf area index of pine plantations on moderate fertility sites will continue to be influenced strongly by pine species even where broadleaved deciduous competition is not controlled. However, leaf area index on high fertility sites will become dominated earlier or to a greater extent by competing broadleaved deciduous species.

Table of Contents

Introduction

Forests contain several long-term carbon storage pools, including the soil carbon and carbon of woody biomass. The rate at which carbon is released back to the atmosphere is controlled by animals, insects, and fungi respiration during the decomposition of the various pools (Brady and Weil, 2007). Although the amount of foliage determine stand-scale photosynthesis, some of which ends up in carbon pools with long residence time, the residence time of carbon invested in foliage (as well as fine roots) is rather short. However, Leaf Area Index (LAI), a unit-less measure of the area of leaves overlaying a given ground area, exerts a strong influence on stand growth by controlling the production of carbohydrates, the photosynthetic chemical reaction that is driven by sunlight absorbed by the leaves (Burton, Zak, Denton and Spurr, 1998). The index is measured in forests to determine the potential sunlight interception and photosynthesis which are related to growth rates.

McCarthy, et al. (2007) assessed the effects CO_2 enrichment had on *Pinus taeda* (L.) (loblolly pine) LAI, showing an increase of LAI under both native and fertilized soil conditions. Results from other experiments showed contrasting responses (Bader, Siegwolf and Körner 2010; Cheng, et al. 2014; Herrick and Thomas 1999, 2003; Liberloo, et al. 2006; Newingham, et al. 2013; Norby, et al. 2003; Riikonen, et al. 2008; Uddling, et al. 2008). Published CO_2-induced enhancement ratios of leaf mass, specific leaf area (SLA) and LAI are presented in the Appendix (Table 1). *Liquidambar styraciflua* (L.) (sweetgum) has been shown to decrease LAI under increasing atmospheric CO_2 (Cheng, et.al. 2014; Herrick and Thomas, 1999, 2003; Warren, Norby and Wellshlager 2011). Other broadleaved deciduous species showed mixed results possibly due to physiological differences between species (Bader, Siegwolf and Körner 2010; Liberloo, et al. 2006; Norby, et al. 2003). Like loblolly pine, *Populus tremuloides* (L.) (American aspen) and *Betula papyrifera* (L.) (American white birch) have shown positive LAI enhancement under increasing atmospheric CO_2, possibly related to increased carbohydrate availability (Newingham, et al. 2013; Riikonen, et al. 2008; Uddling, et al. 2008), with increases associated with higher litter-fall mass but not SLA.

The Free-Air CO_2 Enrichment experimental sites (*FACE*) experiment was designed to provide forest managers and government officials with information regarding the relative capacity of forests to store additional carbon. The focus was on determining forest effectiveness in storing additional carbon under projected increases of atmospheric carbon-dioxide, thus ameliorating anthropogenic CO_2 emissions. One of the *FACE* studies was located in the Blackwood Division of the Duke Forest. The Duke *FACE* experiment was conducted to determine the effects of increasing atmospheric concentrations of CO_2 on tree growth and ecosystem carbon sequestration on relatively infertile sites (McCarthy, et.al. 2007), and where fertility was enhanced through nitrogen (N) fertilization. A +200 ppmv enrichment over ambient atmospheric CO_2 levels during daylight hours was chosen based on increases projected to 2050.

This work examines the effects of elevated atmospheric CO_2 on one major ecosystem property, LAI of planted loblolly pine and broadleaved deciduous species located within the eight 30 m diameter plots of the *FACE* site, utilizing the last two years of 15-17 year-long CO_2 enrichment, and the first two years post enrichment. Given that the broadleaved species replace leaves annually, I hypothesized that their contribution to stand LAI will return to that in unenriched plots the year following the termination of CO_2 enrichment, but that because loblolly pine foliage longevity averages 18 months, its contribution to LAI will take longer to return to ambient. Alternatively, LAI-enhancement may be related to long-term processes of plant hydraulics or nutrient cycling, thus delaying the return to ambient, or to current photosynthesis, and thus hastening the return to ambient LAI to soon after termination of CO_2 enrichment.

Methods

Site description

The Duke FACE study site consisted of loblolly pine planted in 1983. The climate for this region of North Carolina is of warm humid summers and mild winters, mean annual temperatures is 15.8 °C, and annual rainfall average 1145 mm. The soil is of the Enon Series which are low fertility, acidic clay-loam. Pine has predominated the canopy and site, with broadleaved deciduous species comprising the mid- and understory. The most prevalent broadleaved deciduous species on the site is sweetgum.

The experiment was a randomized block design for CO_2 enrichment with 4 paired plots, and a split plot design after nitrogen fertilization was implemented. Nitrogen fertilization was added to determine if increased carbon storage could be achieved on higher fertility sites.

In 1994 the first pair of plots was established, with the remaining three pairs of plots established in 1996 (McCarthy, et.al. 2007). The four control plots remained at ambient carbon dioxide levels, while the four experimental plots received a regulated 200 ppmv increase of carbon dioxide over ambient levels (Oren, 2012). In 1998 a single control plot and experimental plot were split in half with one half receiving annual nitrogen fertilization (11.2 g/m^2 of N). The remaining plots were split in half in 2005 with one half of each plot receiving annual nitrogen fertilization (McCarthy, et.al. 2007). Nitrogen fertilization and carbon dioxide enrichment for all plots was stopped at the end of 2010.

Litter-fall Collection Procedures

Litter-fall was collected monthly from February-September and twice monthly from October-January throughout each year of the study. Some litter collection dates were not met due to inclement weather. The litter-fall was collected using three (0.16 m^2) litter baskets in each plot quadrant and combined into a single (0.48 m^2) sample per quadrant (Oren, 2009a). Litter-fall consisting primarily of pine needles, sweetgum leaves and other broadleaved deciduous leaves combined, was dried (65°C, 4 days), sorted, weighed and recorded.

Calculating Specific leaf area

Subsamples of the litter-fall were measured using the imageJ program (Oren, 2009b) to obtain leaf area, dried and weighed. Specific Leaf Area (SLA cm^2/g) is calculated as leaf area divided by leaf mass based on sub-samples taken at each litter collection time. ANCOVA analysis was conducted in R to compare values across treatments (randomized block design for CO_2 and a split-plot for N fertilization) for each species groups. Sweetgum ($p = 0.0142$) and broadleaved ($p < 0.0001$) differed among CO_2 X N treatments, while loblolly pine did not ($p = 0.138$).

Removal of Fascicle Sheath Mass

Estimating loblolly pine leaf area from mass required the removal of fascicle sheath mass from total pine litter-fall mass. The percentage of fascicle sheath mass for each treatment was calculated by dividing the fascicle sheath mass by the combined fascicle sheath and needle mass from the SLA subsamples. The resultant values were analyzed using ANCOVA in R to test for treatment effects; none were found ($p = 0.3456$). Fascicle sheath percentage was averaged over all treatments and multiplied by total pine litter-fall mass to obtain the needle mass contributing to LAI.

Obtaining Mean Treatment Values

In the fall of 2010, half of each split-plot treatment was harvested, requiring that the post-harvest value of each split-plot treatment is corrected for differences between the harvested and residual portions. The correction was based on relationship between litter-fall mass of the entire plot-half 2009-2010 of each split-plot treatment, and the quarter that was not harvested later. The equations were used to correct 2011-2012 data in order to have a consistent value across years. Once litter-fall for each species was corrected for the effect of the harvest, data were examined for outliers.

<u>Calculating Leaf Area Index</u>

Pine LAI was calculated using Kinerson (1974) and the Sampson (2003) formulas for needle elongation and needle abscission. These formulas provide the relative LAI change for each period; for Kinerson (1974) this is calculated daily, for Sampson (2003) this is calculated monthly. Sampson’s predicted values were interpolated to predict daily values of LAI dynamics for combination with Kinerson’s values. In order to obtain LAI values for the year the relative change were multiplied by the annual needle litter-fall mass. Because needle elongation and abscission rates change among years, the ability to predict LAI depends on how similar the weather of the study years is to that during which the formulas were generated.

Sweetgum and broadleaved species LAI were calculated using the Oren and Pataki (2001) formula for leaf expansion. Broadleaved leaf expansion began after reaching 427 degree days following the last period of four or more days of zero degree days for each year. This varied leaf expansion start dates across the years from March 7th to April 5th. This formula provides the relative leaf expansion calculated over a 30 day period following the methods in McCarthy (2007). Leaf senescence was calculated by removing the litter-fall collections from the total annual litter-fall. The litter-fall values were further modified to predict daily time intervals for LAI change.

Data Analyses

The enhancement ratio of LAI, the ratio by which CO_2 increased LAI in unfertilized and fertilized sites, was calculated for each plot pair by dividing the elevated CO_2 plot LAI by the control plot LAI, for fertilization treatment. The plots at the Duke *FACE* site were paired based on similarity of basal area and soil properties as: 1&2, 3&5, 4&6 and 7&8. One-tailed t-tests were conducted in each of the two enrichment years to assess the significance of a positive CO_2 effect, while post-enrichment years were examined using two-tailed t-tests, as there was an expectation of no effect.

Results and Discussion

Relationships between leaf litter-fall in half-plots and the quarter-plot selected as post-harvest residual based on data from the two years prior to harvest were used to correct for spatial differences. These relationships had R^2 values ranging 0.39-0.99 for sweetgum, 0.62-0.97 for other broadleaved deciduous species, and 0.85-0.98 for loblolly pine.

Fascicle sheath mass represented 0.086 to 0.093 % of the total sample mass. Each species SLA and litter-fall mass (Appendix: Figures A1) were examined intra-annually and inter-annually for trends, as was fascicle sheath mass percent. Loblolly pine SLA and fascicle sheath percent remained consistent across all years, while its litter-fall mass, sweetgum SLA and litter-fall mass, other broadleaved deciduous species SLA and litter-fall mass varied among years. A noticeable decrease in sweetgum and other broadleaved deciduous species SLA was found with doubling of leaf mass per area post enrichment.

Leaf Area Index

Maximum, mean and minimum LAI values (Appendix: Table 2) were calculated on an annual basis for loblolly pine, sweetgum, and other broadleaved deciduous species. Comparing the LAI values for each species in the control plots during periods of CO_2 enrichment and post-enrichment revealed relatively stable LAI for both loblolly pine and sweetgum, while LAI of the other broadleaved deciduous species tended to decline.

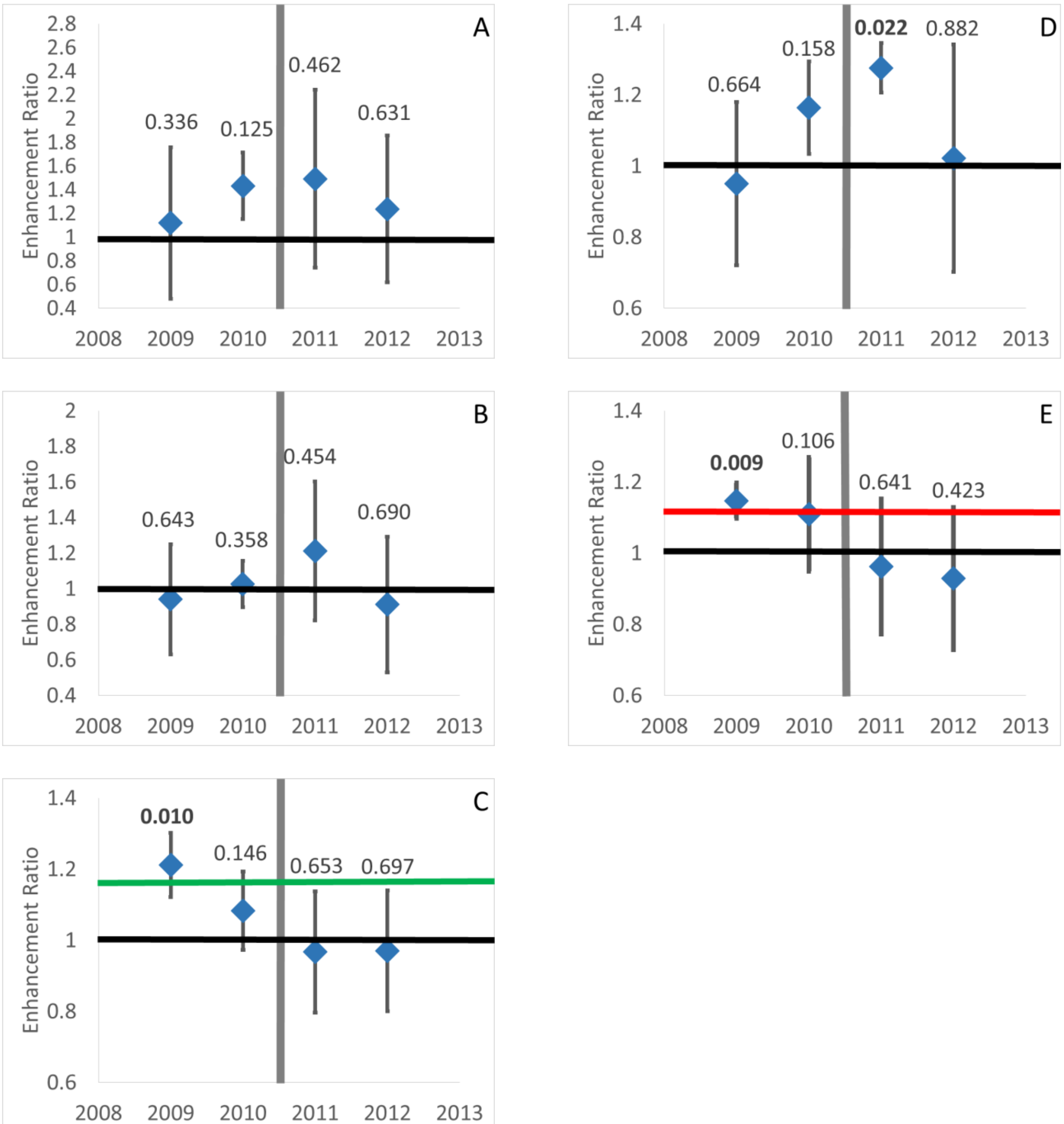
Pine LAI values were increased with CO_2 (Appendix: Table 2). Under elevated CO_2, sweetgum LAI values tended to decrease below those of the ambient conditions in fertilized plots but increased under native soil fertility (Appendix: Table 2). The LAI of other broadleaved deciduous species tended to increase in fertilized plots and decreased when only CO_2 was enriched (Appendix: Table 2).

The enhancement ratio of LAI was significant for loblolly pine and the entire canopy during the enrichment years with increased responses of 15% and 13% on native fertility soils and 7% and 13% in fertilized plots, respectively (Figures 1 and 2), complicated by drought conditions in 2009. The year following the termination of CO_2 enrichment, loblolly pine on both moderate and high fertility plots returned to ambient CO_2 LAI. Total canopy LAI declined below ambient conditions in high fertility plots due to a large broadleaved deciduous component LAI, while in moderate fertility sites broadleaved deciduous species are less dominant.

The LAI enhancement ratio of sweetgum and other broadleaved deciduous species during the two enrichment years did not reveal clear tendencies for Sweetgum or other broadleaved deciduous species regardless of soil fertility (Figures 1 and 2), complicated by the drought in

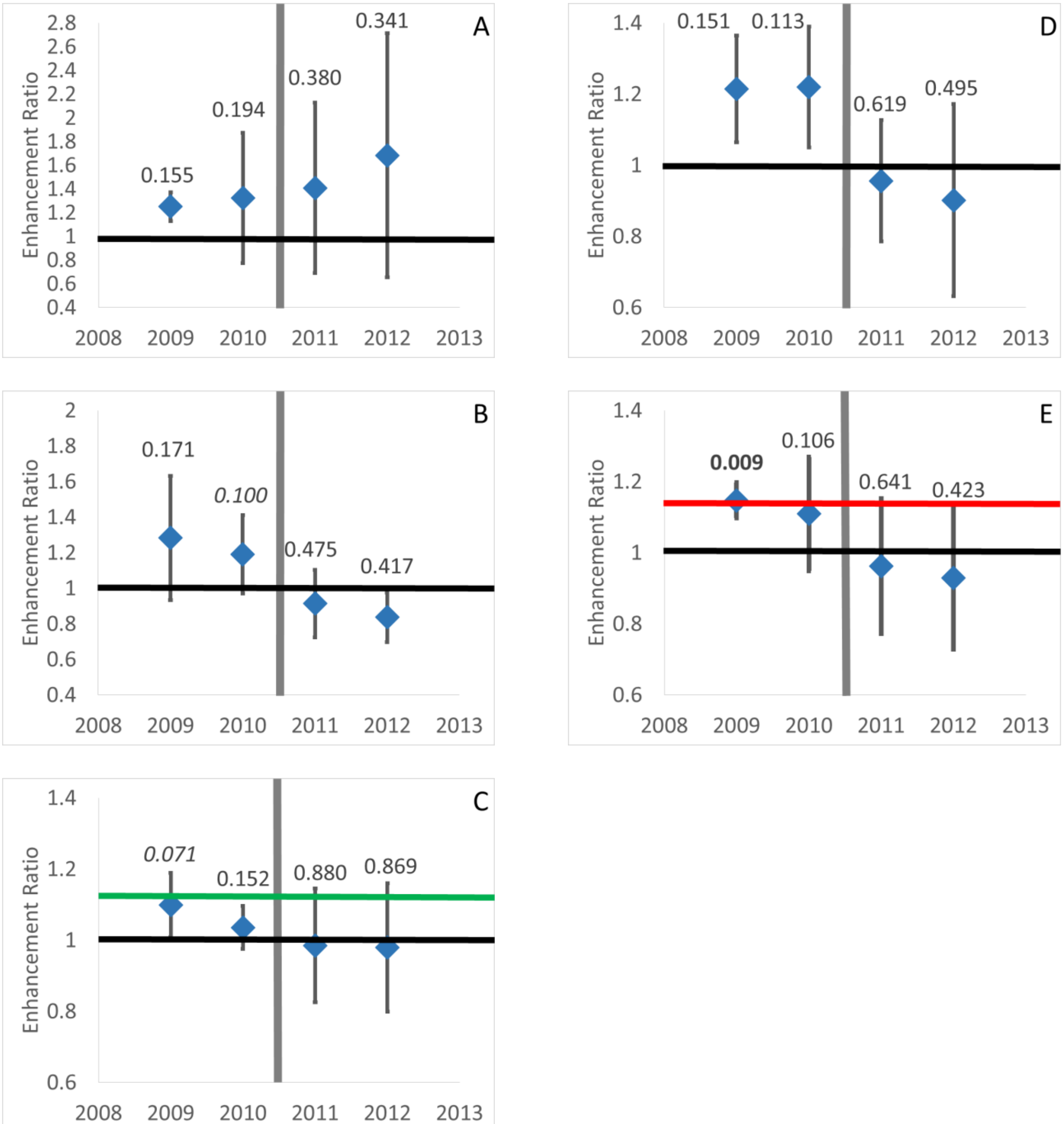
2009, species competition and background variance. These complications are evident in the post-enrichment period for both sweetgum and other broadleaves.

Enhancement Ratio of Leaf Area Index on Moderate Site Fertility



Figures 1: Leaf area index enhancement ratio due to CO_2 enrichment in moderate soil fertility of sweetgum (A), other broadleaved deciduous species (B), loblolly pine (C), combined broadleaved deciduous species (D), and the entire canopy (E). Values shown are P-values from one-tailed t-test for 2009-2010 (Enrichment Period), and two-tailed t-test for 2011-2012 (Post-Enrichment Period). Horizontal green line indicates long-term enhancement for loblolly pine, while horizontal red line indicates the-long term enhancement for the canopy, both from McCarthy, et.al. (2007). Horizontal black lines represent anticipated result for no effect. Error bars represent one standard error.

Enhancement Ratio of Leaf Area Index on High Site Fertility



Figures 2: Leaf area index enhancement ratio due to CO_2 enrichment in high soil fertility of sweetgum (A), other broadleaved deciduous species (B), loblolly pine (C), combined broadleaved deciduous species (D), and the entire canopy (E). Values shown are P-values from one-tailed t-test for 2009-2010 (Enrichment Period), and two-tailed t-test for 2011-2012 (Post-Enrichment Period). Horizontal green line indicates long-term enhancement for loblolly pine, and horizontal red line indicates the-long term enhancement for the canopy, both from McCarthy, et.al. (2007). Horizontal black lines represent anticipated result for no effect. Error bars represent one standard error.

In accordance with the alternative hypothesis, both the contribution of loblolly pine to LAI and LAI of the entire canopy return to that of the ambient conditions the year following the termination of CO_2 enrichment, regardless of soil fertility. Although the data for sweetgum and other species was too variable to be useful for testing the hypotheses, the similarity of the pine and canopy responses, and the fact that broadleaved deciduous species comprised a large proportion of canopy LAI (Appendix: Figure A1) suggest that indeed all species are dependent on currently produced carbohydrates to support higher LAI under CO_2 enrichment. Below I discuss the response of each species and group of species.

Previous results from *FACE* experiments revealed a slight decrease of sweetgum LAI under higher CO_2 (Cheng, et al. 2008, Herrick and Thomas, 1999, 2003; Norby, et al. 2003). This study did not support this pattern (Fig. 1A and 2A). However, sweetgum LAI tended to rise post-enrichment, suggesting that CO_2 enrichment may have held the LAI of this species lower than its potential. Other broadleaved deciduous species did not respond under higher CO_2 on native soil fertility (Fig. 1B and 2B). Under fertilized conditions, however, an insignificant reduction of LAI followed the termination of enrichment. Previous results from *FACE* experiments have corroborated these trends (Bader, Siegwolf, and Körner, 2010; Liberloo, et al. 2006; Riikonen, et al. 2008; Uddling, et al. 2008).

The combination of all broadleaved deciduous LAI reveals two distinct trends based on species interactions for each site. Moderate fertility sites reveal no obvious response to elevated CO_2 (Fig. 1D), suggesting other limiting factors such as nutrient availability or species competition controlled LAI. High Fertility sites showed a tendency for higher LAI of broadleaved deciduous species under enrichment, and a decrease after termination (Fig. 2D).

Effects of carbon dioxide enrichment on loblolly pine showed an increase in LAI on both moderate and high fertility sites, with a 15% and 7% increase, respectively (Fig. 1C and 2C). These results are similar to published responses (McCarthy, et al, 2007; Newingham, et al. 2013), revealing a strong tendency for pine species to increase LAI under increasing atmospheric CO_2. Post-enrichment the LAI values on both sites returned within the year to ambient CO_2 values. The results were complicated by a drought in 2009 which artificially increased pine LAI in 2009 and reduced it in 2010.

Carbon dioxide enrichment increased canopy LAI on moderate and high fertility sites by 13%, similar to previously reported results (McCarthy et al. 2007). The year following termination of enrichment LAI returned to ambient values on both soil fertility settings. The higher availability of carbohydrates in loblolly pine foliage due to increased atmospheric CO_2 allowed lower needles to maintain a positive carbohydrate balance, resulting in increased needle mass (Appendix: Fig. 4F) with crown lengthening. Specific leaf area (Appendix: Fig. A1C) of the pine appears to be relatively constant, revealing limited ability for the species to change their needles morphological shape and construction. In contrast, the main contributor to the response of LAI of sweetgum and other broadleaved deciduous species was not a change of litter-fall, but a decrease of specific leaf area (Appendix: Fig. A1).

Conclusion

Forests on both moderate and high fertility sites in this region should be expected to show a rise in LAI with increasing atmospheric CO_2, with pine continuing as the prevailing component on moderate sites, but broadleaved deciduous species becoming a more dominant component earlier in stand development.

Literary Citations

Bader, M.K., R. Siegwolf and C. Körner (2010). Sustained enhancement of photosynthesis in mature deciduous forest trees after 8 years of free air CO2 enrichment. Planta (232:5).

Brady, N.C. and R.R. Weil. (2007). The Nature and Properties of Soils. 14th ed. Prentice Hall. Upper Saddle River: New Jersey.

Burton, B.V., D.R. Zak, S.R. Denton and S.H. Spurr, (1998). Forest Ecology. 4th ed. John Wiley & sons.

Cheng, L., L. Zhang, Y. Wang, Q. Yu and D. Eamus. (2014). Quantifying the effects of elevated CO2 on water budgets by combining FACE data with an ecohydrological model. Ecohydrology (7:6).

Herrick, J.D., and R.B. Thomas. (1999). Effects of CO2 enrichment on the photosynthetic light response of sun and shade leaves of canopy sweetgum trees (*Liquidambar styraciflua*) in a forest. Tree Physiology (19:12).

Herrick, J.D., and R.B. Thomas. (2003). Leaf senescence and late-season net photosynthesis of sun and shade leaves of overstory sweetgum (*Liquidambar styraciflua*) grown in elevated and ambient. Tree Physiology (23:2).

Kinerson, R.S., K.O. Higginbotham, and R.C. Chapman. (1974). The dynamics of foliage distribution within a forest canopy. Journal of Applied Ecology (11:1).

Liberloo, M., C. Calfapietra, M. Lukac, D. Godbold, Z. Luo, A. Polle, M.R. Hoosbeek, O. Kull, M. Marek, C. Raines, M. Rubino, G. Taylor, G. Scarascia-Mungozza and R. Ceulemans. (2006). Woody biomass production during the second rotation of a bio-energy *Populus* plantation increases in a future high CO2 world. Global Change Biology (12:6).

McCarthy, H.R., R. Oren, A.C. Finzi, D.S. Ellsworth, K. Hyun-Seok, K.H. Johnsen, and B. Millar. (2007). Temporal dynamics and spatial variability in the enhancement of canopy leaf area under elevated atmospheric CO2. Global Change Biology. 13.

Newingham, B.A., C.H. Vanier, T.N. Charlet, K. Ogle, S.D. Smith and R.S. Nowak. (2013). No cumulative effect of 10 years of elevated [CO2] on perennial plant biomass components in the Mojave Desert. Global Change Biology (19:7).

Norby, R.J., J.D. Sholtis, C.A. Gunderson and S.S. Jawdy. (2003). Leaf dynamics of a deciduous forest canopy: no response to elevated CO2. Oecologia (136).

Oren, R. and D.E. Pataki. (2001). Transpiration in response to variation in microclimate and soil moisture in southeastern deciduous forests. Oecologia. 127.

Oren, R. (2009a). Litter Collection Protocol. (See Appendix)

Oren, R. (2009b). Specific Leaf Area Protocol. (See Appendix)

Oren, R. (2012). Leaf Litter Processing Protocol. (See Appendix)

Riikonen, J., K. Kets, J. Darbah, E. Oksanen, A. Sober, E. Vapaavuori, M.E. Kubiske, N. Nelson, and D.F. Karnosky. (2008). Carbon gain and bud physiology in *Populus tremuloides* and *Betula papyrifera* grown under long-term exposure to elevated concentrations of CO2 and O3. Tree Physiology (28:2).

Sampson, D.A., T.J. Albaugh, K.H. Johnsen, H.L. Allen, and S.J. Zarnoch. (2003). Monthly leaf area index estimates from point-intime measurements and needle phenology for *Pinus taeda*. Canadian Journal of Forest Resources. 33.

Uddling, J., R.M. Teclaw, M.E. Kubiske, K.S. Pregitzer and D.S. Ellsworth. (2008). Sap flux in pure aspen and mixed aspen–birch forests exposed to elevated concentrations of carbon dioxide and ozone. Tree Physiology (28:8).

Waring, R.H., P.E. Schroeder, and R. Oren. (1982). Application of the pipe model theory to predict canopy leaf area. Canadian Journal of Forest Research (12).

Warren, J.M., R.J. Norby and S.D. Wellshlager. (2011). Elevated CO_2 enhances leaf senescence during extreme drought in a temperate forest. Tree Physiology (31:2).

Appendix

Table 1: Literature survey of LAI enhancement under increasing CO_2 concentrations. All information was obtained from FACE experiments.

Species	Location	CO2	Condition	Mass	SLA	LAI Enhancement
		ppmv		%	%	%
Broadleaved						
Acer campestre	Bader, et al. (2010)	550			-28.7	
Carpinus betulus	Bader, et al. (2010)	550			4.5	
Fagus sylvatica	Bader, et al. (2010)	550			-4.9	
Liquidambar styraciflua	Cheng, et al. (2014)	560			-2.9	-50
Liquidambar styraciflua	Cheng, et al. (2014)	550			-8	200
Liquidambar styraciflua	Herrick and Thomas (1999)	200+	Sun		30.5	
Liquidambar styraciflua	Herrick and Thomas (1999)	200+	Shade		12.7	
Liquidambar styraciflua	Herrick and Thomas (2003)	200+	Sun		24.2	
Liquidambar styraciflua	Herrick and Thomas (2003)	200+	Shade		8.5	
Liquidambar styraciflua	Norby, et al. (2003)	550		12.5	11.1	4.2
Populus alba	Liberloo, et al. (2006)	550				5.9
Populus fremontii	Newingham, et al. (2013)	550				400
Populus nigra	Liberloo, et al. (2006)	550				8.7
Populus tremuloides	Riikonen, et al. (2008)	560	Spring	-7.1	-4.9	80
Populus tremuloides	Uddling, et al. (2008)	560	Fall			25.8
Betula papyrifera	Riikonen, et al. (2008)	560	Spring	2.2	-5.9	55.6
Populus tremuloides-Betula papyrifera	Uddling, et al. (2008)	560	Fall			31.4
Populus×euramericana	Liberloo, et al. (2006)	550				11.5
Quercus petraea	Bader, et al. (2010)	550			-5.7	
Pine						
Pinus rigida	Newingham, et al. (2013)	550				14.3
Pinus taeda	McCarthy, et al. (2007)	200+	Moderate N			16
Pinus taeda	McCarthy, et al. (2007)	200+	High N			14
Tilia platyphyllos	Bader, et al. (2010)	550			3.5	
Pine and Broadleaved						
Tota Silva canopoeum	McCarthy, et al. (2007)	200+	Moderate N			14
Tota Silva canopoeum	McCarthy, et al. (2007)	200+	High N			12

Table 2: Mean, Minimum, Maximum values of leaf area index (LAI), specific leaf area (SLA), litter-fall mass by year for each treatment. Note the difference between the SLA values and litter-fall mass for loblolly pine and broadleaved deciduous species: the pine has five times lower SLA but four times greater mass. Years 2009-2010 (Enrichment Period), 2011-2013 (Post-Enrichment Period); the pine requires and additional collection year due to needle longevity.

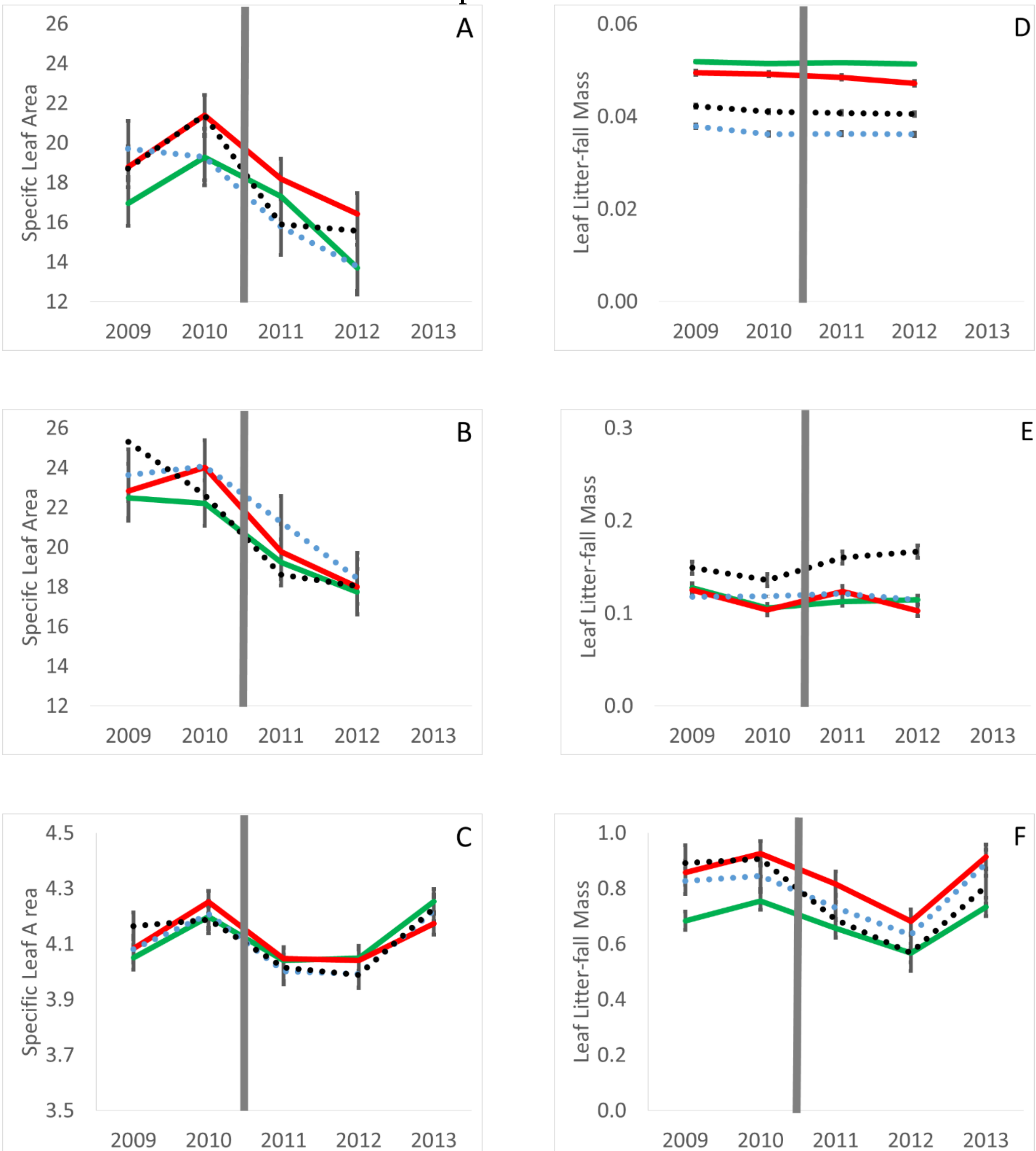
		LAI Mean	LAI Min.	LAI Max.	Specific Leaf Area m2/kg	Litter-fall Mass kg/m2
Sweetgum						
2009	Control	0.56		0.78	16.96	0.052
	Elevated	0.60		0.85	18.80	0.049
	Fertilized	0.38		0.53	19.69	0.038
	Elevated+Fertilized	0.43		0.57	18.69	0.042
2010	Control	0.63		0.83	19.27	0.051
	Elevated	1.02		1.30	21.38	0.049
	Fertilized	0.55		0.70	19.28	0.036
	Elevated+Fertilized	0.80		1.02	21.40	0.041
2011	Control	0.50		0.76	17.30	0.052
	Elevated	0.77		1.04	18.19	0.048
	Fertilized	0.52		0.70	15.78	0.036
	Elevated+Fertilized	0.66		0.88	15.89	0.041
2012	Control	0.59		0.82	13.70	0.051
	Elevated	0.82		1.15	16.43	0.047
	Fertilized	0.44		0.62	13.78	0.036
	Elevated+Fertilized	0.61		0.83	15.57	0.041

Continuation of Table 2 Next Page

Continuation of Table 2

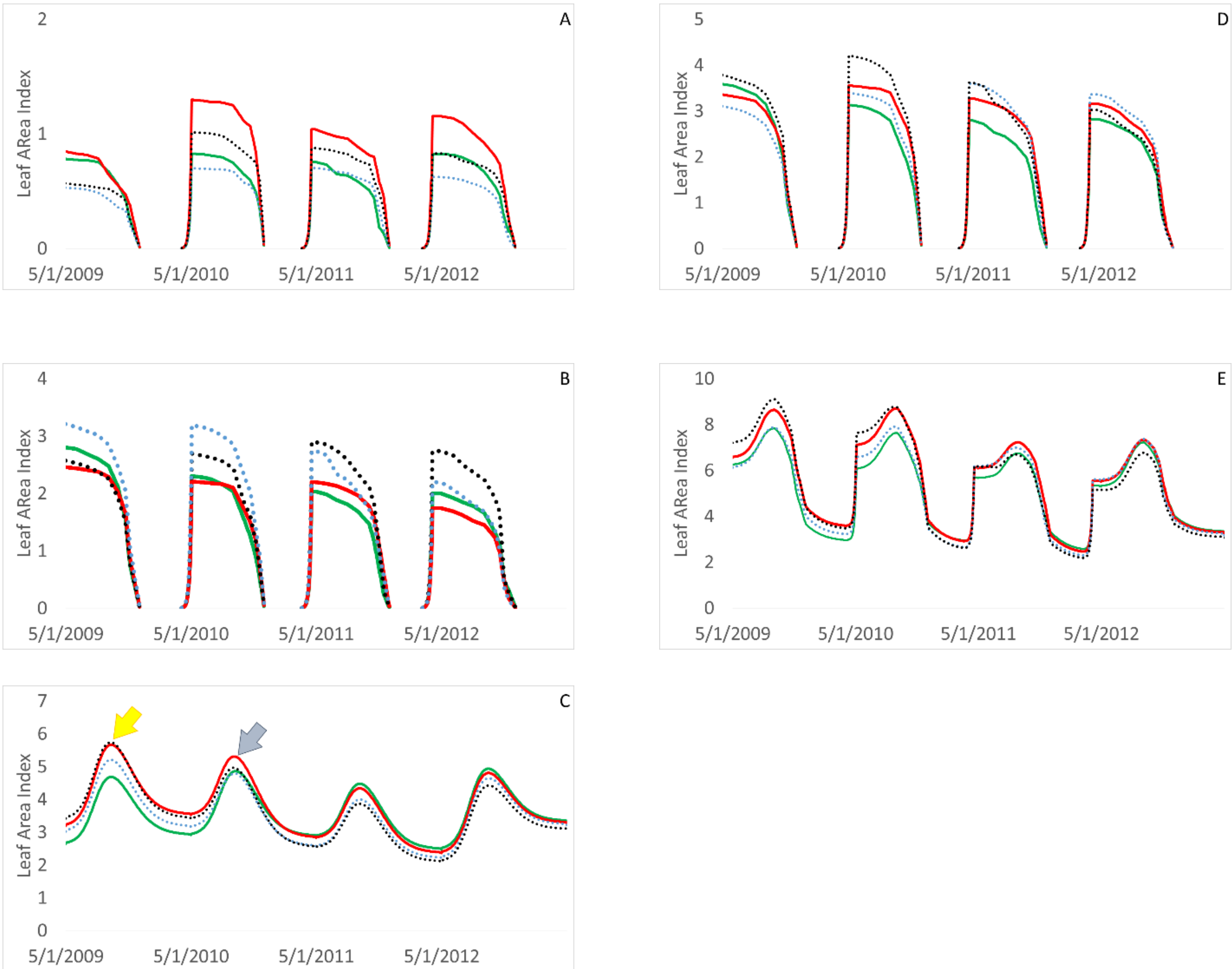
Hardwoods						
2009	Control	2.00		2.80	22.48	0.128
	Elevated	1.84		2.46	22.83	0.125
	Fertilized	1.84		2.57	23.62	0.118
	Elevated+Fertilized	2.34		3.21	25.31	0.149
2010	Control	1.62		2.30	22.21	0.106
	Elevated	1.67		2.21	24.01	0.106
	Fertilized	1.93		2.70	24.08	0.118
	Elevated+Fertilized	2.31		3.19	22.64	0.136
2011	Control	1.40		2.04	19.23	0.113
	Elevated	1.63		2.20	19.77	0.124
	Fertilized	2.01		2.90	21.27	0.121
	Elevated+Fertilized	1.79		2.74	18.61	0.160
2012	Control	1.38		2.00	17.75	0.114
	Elevated	1.21		1.75	18.01	0.103
	Fertilized	1.88		2.74	18.42	0.114
	Elevated+Fertilized	1.43		2.19	18.02	0.177
Pine						
2009	Control	3.55	2.67	4.69	4.05	0.684
	Elevated	4.30	3.17	5.67	4.08	0.857
	Fertilized	3.93	2.96	5.21	4.08	0.755
	Elevated+Fertilized	4.32	3.38	5.74	4.16	0.891
2010	Control	3.66	2.61	4.87	4.20	0.656
	Elevated	3.92	2.83	5.31	4.25	0.926
	Fertilized	3.55	2.52	4.80	4.21	0.567
	Elevated+Fertilized	3.64	2.54	4.97	4.19	0.907
2011	Control	3.33	2.48	4.48	4.04	0.827
	Elevated	3.22	2.35	4.34	4.05	0.817
	Fertilized	2.97	2.21	4.00	4.00	0.845
	Elevated+Fertilized	2.87	2.10	3.87	4.01	0.687
2012	Control	3.79	2.52	4.94	4.05	0.732
	Elevated	3.70	2.41	4.81	4.04	0.682
	Fertilized	3.50	2.26	4.65	3.99	0.633
	Elevated+Fertilized	3.41	2.14	4.43	3.99	0.568
2013	Control				4.25	0.733
	Elevated				4.17	0.914
	Fertilized				4.22	0.892
	Elevated+Fertilized				4.23	0.809

Inter-Annual Variation of Specific leaf area and Litter-fall Mass



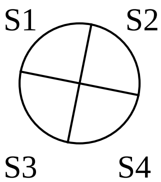
Figures A1: Inter-annual variation of Specific leaf area (SLA) and Litter-fall Mass of sweetgum (A), other broadleaved deciduous species (B), and loblolly pine (C). Green line (Control), red line (elevated CO_2), black line (fertilized), blue line (fertilized + elevated CO_2). Note the difference between the SLA values and Litter-fall Mass for pine and broadleaved deciduous species, pine has five times lower SLA but four times greater mass. Years 2009-2010 (Enrichment Period), 2011-2013 (Post-Enrichment Period), Pine requires and additional collection year due to needle longevity.

Seasonal Dynamics of Leaf Area Index



Figures A2: Seasonal dynamics of leaf-area index (LAI) of sweetgum (A), other broadleaved deciduous species (B), loblolly pine (C), combined broadleaved deciduous species (D), and the entire canopy (E). Green line (Control), red line (elevated CO_2), black line (fertilized), blue line (fertilized + elevated CO_2). Years 2009-2010 (Enrichment Period), 2011-2013 (Post-Enrichment Period). Due to methodological artifact, pine LAI in Pine 2009 (Yellow Arrow) is overestimated, while that of 2010 (Purple Arrow) is underestimated.

LITTER COLLECTION (Oren, R. (2009a))



Litter is collected on or near the first day of each month, and twice a month in the fall (**Feb-Sep**: monthly, **Oct-Jan**: twice monthly). There are 3 litter baskets (laundry baskets) in each sector/quadrant of every ring. The litter from all 3 baskets is combined into one collection bag. There are 2 twig collectors (wooden baskets) in each ring, one in sector 2 and one in sector 4. The twigs and bark are collected into one bag for each sector.

Preparation: Label bags with date, ring and sector (Dec. 16 R1S3); n = 48 litter bags. Also label bags for twig collection (Dec. 16 R1S2 TWIG); n = 24 bags.

Litter baskets: Collect all litter within the perimeter of the basket and place into the bag. If there are large branches, cut them and collect only the portion that was resting on the basket.

Twig collectors:
Place all twigs and branches into the collection bag. Collect all pieces of bark equal to or larger than the size of a quarter. If needles are attached to twigs, remove them. Remove all other litter collected in the basket and place underneath the basket.

Once the litter collection is finished, keep the litter bags in the cold room until sub-samples for specific leaf area are taken. After sub-samples have been taken, the litter bags and twig collection bags must be put in the drying oven for 3 days before they are processed. Litter bags are sorted and weighed with the help of student assistants. Twig collector bags are simply separated into pinewood and hardwood and then weighed. See “Litter Sorting” section below.

SPECIFIC LEAF AREA (SLA) (Oren, R. (2009b))

Processing SLA Subsamples

1. Litter from the field will be brought into the lab. Immediately following, subsamples will be taken. These sub-samples will consist of 5 needles/leaves which are in good condition. The sub-samples will be put in the cold room while the rest of the sample will be put in the lab to air dry until sorted.
    a. **Feb-Aug** sub-samples will consist ONLY of pine.
    b. **Sep-Jan** sub-samples will consist of pine, sweetgum, and other hardwoods.
2. Specific leaf area (SLA) will then be determined for each category. For pine needles, fascicle sheaths must be removed before the SLA is taken. Review specific leaf area protocol for details.
3. Once SLA is taken, samples should be put in the drying oven for at least 3 days.
4. After samples are dry, each sample should be weighed. For the pine needles, the needle and fascicle sheaths should be weighted separately. Record weights on the data sheet. Enter data onto computer network: \\Alatus\Shared space\Lab data\Face\Litter\SLA…

Analyzing SLA Subsamples

1. Cut off the bundle sheath from the needles. Line up the needles so that they do not touch each other on the flatbed scanner. In each image, include something of known length so the scale can be set in the imaging program.
2. Open the Epson Scan program.
    a. Make sure of the following settings:
        i. Mode = Professional Mode
        ii. Document Type = Reflective
        iii. Document Source = Document Table
        iv. Auto Exposure Type = Document
        v. Image Type = 8-bit Greyscale
        vi. Resolution = 600dpi
        vii. Target Size = Original
    b. Click Preview
    c. With the mouse, draw a box just over the area you want to scan (this will keep the file size smaller and scan time less)
    d. Click Scan
    e. The file will automatically be placed in the Scans folder on the desktop
3. Open the ImageJ program.
4. Set scale:
    a. Draw a line on the scanned scale.
    b. Go to the Analyze menu and select ‘Set Scale’
    c. Fill in the known distance of the scanned scale. Make sure the units are correct for the scale you are using. Note: ‘Global’ should not be checked.
    d. Click Ok.
5. Delete anything you do not want the program to measure.
    a. Draw a box over the object you want to delete and then hit Cntl-X.
        i. Should delete the scanned scale

        ii. Should delete any non-leaf material that was scanned
    b. Draw additional squares and repeat if needed.
    c. Click anywhere on the image to get rid of the square.
6. Setting the threshold (on a clean image):
    a. Go to the Image menu, select ‘Adjust’, and then ‘Threshold’
    b. Note: if the image is not clean – go to the Process menu and select ‘Subtract Background’
    c. Adjust the slide bars so that the red covers the area you want to measure.
    d. All boxes should be checked. Click Apply. Click Ok.
7. Measuring the area:
    a. Go to the Analyze menu and select Analyze Particles
    b. The following boxes should be checked:
        i. Display Results
        ii. Clear Results
        iii. Summarize
    c. Click Ok.
8. A window will show up and the Summary value is the SLA value.
9. Go to the File menu and click ‘Save as’ to save the summary results window.

LITTER SORTING (Oren, R. (2012))

There are 4 litter bags to sort for each ring (1 per sector). Every ring is divided into a control half and a fertilized half. The sectors in each half vary in different rings. Use the chart in the lab to look at the setup for each ring (see below). The 2 bags for the control half are sorted, weighed, and the litter from each bag is combined and placed into envelopes for the Finzi lab. The same is done for the 2 bags for the fertilized half. The data sheets for recording should be printed out before each litter collection is sorted. They can be found on the network: \\Alatus\Shared space\Lab data\Face\Litter\Litter_Datasheets. Change the date on each sheet to the date that the litter collection was made. Put the sheets in the folder kept in the sorting area.

Quadrant				
Plot	**Control**		**Fertilized**	
1	1	4	2	3
2	3	4	1	2
3	1	4	2	3
4	1	2	3	4
5	3	4	1	2
6	3	4	1	2
7	3	4	1	2
8	2	3	1	4
9	1	2	3	4
10	1	2	3	4
11	1	4	2	3
12	3	4	1	2

Sort samples into:

- Pine Needles
- Sweetgum Leaves
- Other Hardwood Leaves
- Pine Wood (bark & branches)
- Hardwood Wood (bark & branches)
- Cones (catkins, pine cones, or pine cone pieces)
- Pine Seeds
- Hardwood Reproductive (other seeds, flowers, petals)
- Debris
- Juniper

Seed/Cone Counting

- Seeds and catkins are counted and recorded on the data sheet.
- Sort pine seeds into immature, predated, and whole seeds.

    - Count immature pine seeds
    - Count predated seeds (2 empty shells = 1 predated seed).
    - Count mature pine seeds. The weights for individual seeds will be recorded on a separate data sheet when the rest of the litter is weighed.
- Count pollen cones (catkins). Estimate entire cones (2 parts = one cone).
- Sort and separate other seeds. Identify what species they are using the samples in the seed key box and record on the data sheet (elm, dogwood, poplar, sweetgum, cherry).

Weighing

- Weigh each mature pine seed and record the weight on the data sheet.
- Weigh the sorted litter for each category and record on the data sheet.
- Label coin envelopes with the date, ring, control/fertilized, “Oren/Finzi”, and one of the following categories:

  Pine Needles
  HW Leaves
  Pine Wood
  HW Wood
  Pine Reproductive
  HW Reproductive
  Debris

  Label ex:

  5-1
  R1 Control
  Pine Needles
  Oren/Finzi

- Sweetgum and other hardwood leaves are combined into the envelope for HW Leaves. Pine Reproductive includes pine seeds, cones, and catkins. HW Reproductive includes acorns, hardwood seeds, and flowers. Juniper is put into the envelope for debris. Put about 10g of each sample into the envelope. If there is less than 10g, add the entire sample.
- For pine needles, if there is more than 10g, label an envelope with date, ring, control/fertilized, contents, and “Oren Archive”. Store these envelopes in a separate box in the lab.
- Any leftover material can be discarded.
- After one litter bag has been processed, sort the second bag for that fertilized or control group, weigh it, and add those samples to the envelopes. Place the envelopes in a bag labeled with the date, ring, control/fertilized, and “Oren/Finzi”. The bags go into a box that will be mailed to the Finzi lab. If you only have time to sort and weigh one bag, just leave the envelopes on the lab bench with the second bag beside them.

- Enter the data for seeds and litter weights on the network. \\Alatus\Shared space\Lab data\Face\Litter\Shannonseeds… (sheet named seedfile) and \\Alatus\Shared space\Lab data\Face\Litter\Litter_Data…